at least the N-terminal region of a truncated mature Fha protein.

REMARKS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

At the outset, the undersigned wishes to express appreciation to the Examiner and her supervisor for the very helpful telephonic interview of January 30, 2003. Once the Examiner's Summary is in hand, further comments regarding the interview will be provided, if necessary.

Claims 1, 7, 34 and 40 have been revised to define the invention with additional clarity (and to correct minor clerical errors) and new claim 42 has been added. The new claim does not raise new issues as it corresponds to claim 40 prior to the present amendment of claim 40.

Claims 1-15, 18-22, 27-30 and 39 stand rejected under 35 USC 103(a) over Loosmore et al in view of Menozzi et al. Claims 34, 35 and 37 stand rejected under 35 USC 103(a) over Loosmore et al in view of Menozzi et al and Locht et al. The rejections are traversed for the reasons that follow.

At the outset the Examiner is reminded that the recombinant DNA of the present invention comprises two components:

i) the first is a sequence that encodes a polypeptide heterologous with respect to a filamentous hemagglutinin of *Bordetella* (Fha), and

ii) the second is a sequence that encodes the site of FHA that interacts with heparin.

The claims require that the second of these sequences be upstream of (and in the same reading frame with) the first so that the FHA/heparin interaction site is N-terminal to the heterologous polypeptide in the resulting fusion protein.

Turning to the rejection, the Examiner again takes the view that Loosmore et al discloses fusion proteins that comprise an amino acid sequence from Fha fused to an amino acid sequence from a protein distinct from Fha. Respectfully, it is believed that the Examiner's continued reliance on Loosmore results from some confusion over the distinction between a <u>transcriptional fusion</u> (wherein nucleoside sequences from two different origins are linked but do not result in a hybrid open reading frame encoding a fusion protein) and a <u>translational fusion</u> (wherein the gene fusion can be translated into a fusion protein

encompassing two amino acid sequences from different origins). (The present invention is of the latter type.)

In rejecting the claims as obvious, the Examiner directs attention to the gene fusions Fhap/TOX, Fahp/PRN and TOXp/Fha taught by Loosmore. Applicants point out, however, that these gene fusions do not code for "fusion proteins comprising an amino acid sequence from Fha fused to an amino acid sequence from a protein distinct from Fha". Rather, and as explained by Loosmore, these fusions were obtained "by fusing the promoters with the structural genes at the ATG start codon of the structural gene" (column 4, lines 34-36). The next sentence of Loosmore specifies that "[s]uch fusions result in a native but autologous promoter, and a structural gene with its natural signal sequence" (emphasis added).[1]

The fusions described by Loosmore thus comprise a nucleotide sequence from the Fha gene (i.e., the promoter sequence), and a nucleotide sequence from a gene heterologous thereto (i.e., the sequence encoding a structural gene such as TOX). However, as described unambiguously in Loosmore, the gene fusions taught do not

[1] As pointed out previously, in the names of the gene fusions mentioned above, the letter "p" stands for "promoter". For example, Fhap/TOX designates a fusion between the Fha promoter and the TOX coding sequence.

encode a fusion protein comprising and amino acid sequence from Fha fused to an amino acid sequence from a protein distinct from Fha (as required by the claims).

It has been acknowledged that Menozzi et al teach that the Fha protein is able to interact with heparin. However, Applicants reiterate that one skilled in the art reading this article and the application by Loosmore et al would not have arrived at the claimed invention, since the Loosmore et al reference is not relevant, for the reasons detailed above.

The rejection of claims 34 , 35 and 37 over Loosmore et al in view of Menozzi et al and Locht et al is again traversed since, given the fundamental failings of the primary reference discussed above, there is nothing in Locht et al that would have suggested making fusion proteins with an Fha moiety.

Reconsideration is requested.

Claims 40 and 41 stand rejected under 35 USC as allegedly being anticipated by Relman et al. The rejection is traversed.

Relman et al describes the purification of large amounts of Fha fragments. To that end, the FhaB open reading frame was divided into seven fragments that were separately cloned into the expression vector pEX34, which

comprises a promoter and a sequence encoding the N-terminal 98 amino acids of the MS2 RNA polymerase. This is explained, for example, at column 9, lines 45 to 59 of Relman.

The Examiner contends that Relman et al teaches fusion proteins having a N-terminal moiety consisting of an Fha N-terminal fragment (the Examiner directs attention to column 2, lines 30-35, and claims 8-10). However, the cited paragraph of column 2 merely describes the Fha ORF, specifying that it comprises a N-terminal fragment of 230 kDa, and the indicated claims recite nucleic acids encoding the amino-terminal 313 kDa of the Fha protein (claims 8 and 9), and an expression construct comprising this nucleic acid operably linked to other nucleic acids comprising transcription initiation and termination regions (claim 10). None of the claims to which the Examiner refers describes or would have suggested the construction of a fusion protein comprising a N-terminal moiety originating from Fha. Applicants submit that the fusion proteins described by Relman et al all comprise a N-terminal moiety from MS2 and a C-terminal moiety corresponding to a fragment of FHA. Although one fusion comprises the N-terminal extremity of Fha, this fragment is

still in the C-terminal portion of the corresponding fusion protein.

In the context of the present invention, the presence and location of the N-terminal part of Fha (comprising the Fha/heparin interaction site) in the fusion protein are important as it is this part that contains the secreting determinant of the Fha. This determinant must be located in the fusion protein N-terminal to the heterologous polypeptide (as required by the instant claims) for secretion or cell surface presentation to be effected. (For the Examiner's information, the function of the N-terminal part of the protein is discussed in Jacob-Dubuisson et al, Molecular Microbiology 40:306-313 (2001) - a copy of which is provided for the Examiner's ease of reference).

In considering the teachings of Relman et al, it is important to bear in mind that, as indicated above, the focus of that citation was the production by *E. coli* of Bordetella proteins, not an easy task given the high GC content of the corresponding genes. To overcome the difficulties faced, Relman et al fused the gene (or portion thereof) encoding the Bordetella protein to be produced (Fha) with the 5' part (or N-terminal encoding portion) of the gene of a protein produced efficiently by *E. coli* MS2.

The fusion protein was thus produced in *E. coli* but not secreted.

Enclosed is an article by Domenighini, Relman and others, describing the same fusion proteins as those mentioned in USP 6,036,960. In this article, the authors refer to a publication by Strebel et al (1986), apparently as describing pEx34 (see page 794, second paragraph of left column of Domenighini et al). The article by Strebel et al is also enclosed. It will be noted that Strebel et al does not recite exactly a "pEx34" plasmid, but that it describes several expression vectors for the construction of fusion proteins, designated "pPLc24", "pEx30", "pEx31" and "pE34". All of these vectors are designed to construct fusion proteins in which the N-terminal moiety consists of 98 N-terminal amino acids of the MS2 polymerase. This appears clearly in the last paragraph of page 984 (pE34 vector) and Figure 1 (pEx30 and pEx31).

An article by Nicosia et al (1987) is enclosed that explicitly mentions the pEx34 vector. In this article, pEx34 is defined as a derivative of pEx29, together with pEx31 (see first paragraph of Materials and Methods). To describe these vectors, Nicosia et al refers to the Strebel reference described above and to another article by Klinkert et al (see the last paragraph of page 964 of

Nicosia et al for this reference). Klinkert et al (1985, copy of which is also enclosed) describes the pEx29 expression vector and it appears clear, from paragraph 2 of page 230 and from Figure 2, that the fusion proteins obtained using this vector comprise a fragment of the MS2 protein as their N-terminus.

In view of the above, it will be clear that the fusion proteins described in the cited Relman patent have a N-terminal moiety from MS2 and a C-terminal moiety from Fha. Hence, the Examiner's assertions to the contrary, the citation does not anticipate the subject matter of the rejected claims. Reconsideration is requested.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached pages are captioned "**Version With Markings To Show Changes Made**."

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

1. (Thrice Amended) A recombinant DNA encoding an immunogenic fusion protein, wherein said recombinant DNA comprises [comprising] a sequence (1) coding for a polypeptide heterologous with respect to a filamentous hemagglutinin of *Bordetella* (Fha) fused in the same reading frame with a sequence (2) placed upstream from said sequence (1), said sequence (2) coding for at least a part of the precursor of the Fha, this part comprising [at least the N-terminal region of a truncated mature Fha protein which contains] the site of interaction of the Fha with heparin [, said sequence (2), when placed under the control of a promoter recognized by the cellular polymerases of *B. pertussis* and introduced into a *B. pertussis* cell culture is expressed in this culture and excreted into the culture medium of these cells or exposed at the surface of these cells, wherein said recombinant DNA when expressed produces highly immunogenic fusion proteins].

7. (Four Times Amended) The recombinant DNA according to claim 4, wherein the extension of the sequence

(2) towards its C-terminus will not exceed the length which would cause the transformation of *B. pertussis* with this recombinant DNA then placed under the control of a promoter capable of being recognized by *B. pertussis* to [on] no longer permit the direct excretion of the recombinant protein then formed into the culture medium of this *B. pertussis*.

34. (Amended) A recombinant DNA comprising a sequence (1) coding for a polypeptide heterologous with respect to a filamentous hemagglutinin of *Bordetella* (Fha) fused in the same reading frame with a sequence (2) placed upstream from said sequence (1), said sequence (2) coding for at least a part of the precursor of the Fha, this part comprising [at least the N-terminal region of a truncated mature Fha protein which contains] the site of interaction of the Fha with heparin, [said sequence (2), when placed under the control of a promoter recognized by the cellular polymerases of *B. pertussis* and introduced into a *B. pertussis* cell culture is expressed in this culture and excreted into the culture medium of these cells or exposed at the surface of these cells,] wherein the resulting fusion protein [being able to facilitate] facilitates the

presentation of the antigen encoded by the heterologous sequence (1) to the mucosal immune system.

40. (Amended) A culture of bacterial cells belonging to a bacterial species other than *Bordetella* and transformed by a recombinant DNA comprising a sequence (1) coding for a polypeptide heterologous with respect to a filamentous [hemagglutitin] hemagglutinin of *Bordetella* (Fha) [and having vaccinating properties against a given pathogenic agent], said sequence (1) being fused in the same reading frame with a sequence (2) placed upstream from said sequence (1), said sequence (2) coding for at least a part of the precursor of the Fha, this part comprising [at least the N-terminal region of a truncated mature Fha protein which contains] the site of interaction of the Fha with heparin.